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GENETIC AND PHYSIOLOGICAL ANALYSIS OF MITOMYCIN C-SENSITIVE MUTANTS OF *ESCHERICHIA COLI* K12

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SUMMARY Mutants of *Escherichia coli* which were mitomycin C (MTC) sensitive and ultraviolet light (UV) insensitive were divided into two groups. One group was controlled by a gene (*mtcA*) located close to the *lac* gene (Otsuji, 1968), and the other by a gene (*mtcB*) located at about 58 min on *E. coli* chromosome. Mutation of *mtcA* as well as *mtcB* has a pleiotropic effect; the mutants are sensitive to MTC, basic dyes, sodium dodecylsulfate and sodium deoxycholote. The *mtcA* mutants and wild type bacteria are sensitive to colicin El, E2, E3 and K, while the *mtcB* mutants are resistant to colicin E1, and sensitive to E2, E3 and K. The increased sensitivity to basic dye of the mutants is due to increased incorporation of the dye into the cell. ^{14}C-Proline uptake by membrane preparations of wild type bacteria was inhibited at a lower concentration of colicin E1 than uptake by those of *mtcB* mutants. From the results we concluded that the *mtcB* mutants have an altered cell surface which renders the cells sensitive of MTC and insensitive to colicin E1. Genetic and physiological analyses of the *mtcB* mutants suggested that the *mtcB* gene may be identical to the *tolC* gene described by Whitney (1971) and by De Zwaig and Luria (1967).

INTRODUCTION

There is evidence that the effects of mitomycin C (MTC) and ultraviolet light (UV) are similar (Boyce and Howard-Flanders, 1964; Greenberg et al., 1961; Iyer and Szybalski, 1963; Otsuji, 1968; Otsuji et al., 1959; Otsuji and Okubo, 1961; Shiba et al., 1959; Szybalski and Iyer, 1967). In the previous paper, however, we (N.O.) reported that a class of mutants, which were sensitive to MTC, but insensitive to UV, were controlled by a gene, *mtcA*, located close to the *lac* gene of the *E. coli* chromosome and these mutants did not have a defect in the mechanism for repairing cross-links between DNA strands (1968). This paper reports that another class of MTC-sensitive, UV-insensitive mutants is controlled by a mutation of the *mtcB* gene located at about 58 min on the *E. coli* chromosome. Experimental results suggest-

ing altered permeability or an altered surface structure in the mutant strains are also presented.

MATERIALS AND METHODS

1. *Bacterial strains*

The strains used in this experiment are listed in Table 1. Strains M5, M11, M18, M27, and M28 are MTC sensitive, UV insensitive mutants which were isolated from AB1157, after treatment with N-methyl-N′-nitro-N-nitrosoguanidine in Osaka University (Otsuji, 1968).

2. *Media*

The compositions of M buffer, minimal (M) medium, enriched M (EM) medium and nutrient broth (NB) medium are described elsewhere (Otsuji, 1968). Tryptone broth (TB) medium contained 10 g of tryptone (Difco), 5 g of yeast extract, 5 g of NaCl, 1 g of glucose, and water to 1 liter. The pH was adjusted to 7.2 with NaOH. Medium for recombination (RC) contained (per liter): 10 g of tryptone, 10 g of NaCl, 5 g yeast extract, 8.7 g of K_2HPO_4, 6.8 g of KH_2PO_4, 1 g of NH_4Cl, 0.1 g of $MgSO_4$, 5 mg of $CaCl_2$ and 0.25 mg of $FeSO_4 \cdot 7H_2O$. The pH was adjusted to 6.3.

3. *Recombination*

Cells used for mating were grown in RC medium to the log phase (about 3×10^8 cells/ml) from an inoculum from an overnight culture. Volumes of 0.1 ml of the male strain and 1 ml of the female strain were mixed in a test tube. The mixture was incubated at 37 C with reciprocal shaking (60 strokes/min) and plated on a selective agar medium. To determine the frequency of unselected donor markers among the recombinants, about 100 recombinants were purified on NB plates. Single colonies of each recombinant were transferred to further NB plates and then replica plating was done on appropriate agar media.

In F-duction experiments, strains were mixed in a test tube, using 0.1 ml of the male strain and 1 ml of the female strain, or 1 ml of the male and 0.1 ml of the female strain. Incubation was carried out at 37 C for 60 min with reciprocal shaking (120 strokes/min).

TABLE 1. *Bacterial strains used*

Strain	Mating type	Relevant genotype	Other markers	Source
AB1157	F⁻	+	*thr leu arg his pro ara lac gal mtl str tsx thi*	Howard-Flanders
AB1185	F⁻	+	*thr leu arg his pro ara lac gal mtl str tsx thi uvrB*	Howard-Flanders
M5	F⁻	mtcB5	*thr leu arg his pro ara lac gal mtl str tsx thi*	AB1157 by NG
M11	F⁻	mtcB11	*thr leu arg his pro ara lac gal mtl str tsx thi*	AB1157 by NG
M17	F⁻	mtcA17	*thr leu arg his pro ara lac gal mtl str tsx thi*	AB1157 by NG
M27	F⁻	mtcA27	*thr leu arg his pro ara lac gal mtl str tsx thi*	AB1157 by NG
M28	F⁻	mtcA28	*thr leu arg his pro ara lac gal mtl str tsx thi*	AB1157 by NG
BE995	F⁻	mtcB11	*arg his pro ara lac gal mtl str tsx thi*	HfrH×Ml 1
BE591	Hfr	+	*phoA trp lam*; injection order: *purE leu thr*	
AB312	Hfr	+	*thr leu lac thi str*; injection order: *fda argG str*	Adelberg
AB313	Hfr	+	*thr leu lac thi str*; injection order: *tna xyl str*	Adelberg
S 21-1-1	Hfr	+	*thr leu thy ilv thi*; injection order: *lys thyA his*	Nishimura
S 3-1-1	Hfr	+	*thr leu thy ilv thi*; injection order: *fda argG str*	Nishimura
AT2446	Hfr	+	*thi metC69*	A. L. Taylor

Abbreviations; The symbols *arg*, *his*, *ilv*, *leu*, *pro*, *trp*, *thi*, *thr*, *thy* and *pur* denote requirements for arginine, histidine, isoleucine and valine, leucine, proline, tryptophan, thiamine, threonine, thymine, and purine respectively; *ara*, *gal*, *lac*, *mtl*, *xyl*, denote the inability to utilize arabinose, galactose, lactose, mannitol, and xylose, respectively; *tsx*, *lam* and *str* denote response to the phage T6, λ and to streptomycin (r, resistant; s, sensitive); *mtc* denotes gene affecting mitomycin sensitivity; *colE1*, *colE2 colE3* and *colK* denote producers of colin E1, E2, E3 and K, respectively. The symbols *fda*, *phoA* and *tna*, denote genes for fructose-1-6-diphosphate aldolase, alkaline phosphatase and tryptophanase.

4. *Pl transduction*

Recipient bacteria were grown in TB medium containing 2.5×10^{-3} M $CaCl_2$ to about 2×10^8 cells/min. Then they were resuspended in 1/5 volume of the same medium and Plvir phage was added at a multiplicity of infection of about 0.05. Adsorption was completed by incubating the mixture for 20 min at 37 C. Then the mixture was centrifuged and the pellet was resuspended in 0.85% NaCl and an aliquot was plated on a selective agar plate.

5. *Drug survival curve*

To determine the survival of bacteria, cultures were first grown at 37 C with shaking in EM medium to a density of about 3×10^8 cells/ml. They were then centrifuged, washed once and resuspended in M buffer. Then, they were exposed to various concentrations of the chemicals to be tested for 30 min at 37 C. After appropriate dilution, samples were plated on NB plates and incubated for about 20 hr at 37 C.

6. *Preparation of colicin plates*

An overnight culture of colicinogenic bacteria was harvested by centrifugation, washed once and suspended in one tenth volume of saline. A 0.1 ml sample of the culture was plated on an NB agar plate and incubated at 37 C for about 30 hr. Cells were killed with chloroform vapor and 6 ml of melted NB agar (1.5%) were poured onto the surface of the plate.

7. *Preparation of colicins*

An overnight culture of colicinogenic bacteria was diluted 20 fold in NB broth (100 ml) and grown to about 3×10^8 cells/ml. The cells were harvested, suspended in 10 ml of saline and irradiated in a petri dish for 45 sec (Toshiba germicidal lamp, 15 w, 45 cm distance). The cells were diluted to 200 ml with M medium containing 1% tryptone and aerated for 3.5 hr at 37 C. After centrifugation, cells were suspended in 2 ml of M buffer and ruptured by sonic disintegration (Kaijo Denki Ultrasonic Disintegrator, Model TA-4201, 20 KC, 2 min) in an ice-water bath. The suspension was centrifuged at 15,000 rpm for 20 min to remove debris and the resulting supernatant fraction was used as the colicin preparation. The colicin titer was about 3×10^{11} killing particles/ml.

8. *Assay of binding of methylene blue to bacterial cells*

Binding of methylene blue to bacterial cells was determined by as described by De Zwaig and Luria (1967). Bacteria grown to the log phase in EM medium were harvested, washed once and suspended in M buffer. Then, methylene blue was added at a concentration of 10 μg/ml and the mixture was incubated at 28 C with reciprocal shaking (60 strokes/min). At intervals, 2 ml portions of the cells were removed and filtered through Millipore HA filters (0.45 μ). The filters were soaked in 3 ml of M buffer and 0.75 ml of a 25% solution of SDS was added. The samples were incubated at 45 C and the optical density of the clear solution thus obtained was measured in a Hitachi 124 Spectrophotometer at 660 mμ.

9. *Preparation of membranes*

Bacteria grown in 2 liter of NB broth to the logarithmic phase were centrifuged, washed twice with 0.1 M tris buffer, pH 8.0 and resuspended in 10 ml of 0.03 M tris buffer, pH 8.0 containing 0.5 M sucrose. EDTA and lysozyme (Worthington, crystalline) were added to final concentrations of 5×10^{-3} M and 500 μg per ml, respectively. The mixture was stood for 30 min in an ice-water bath, mixed with $MgSO_4$ at a final concentration of 0.05 M and centrifuged at 10,000 rpm for 10 min. The precipitate was suspended in 3 ml of 0.1 M potassium, phosphate buffer, pH 6.6, containing 20% sucrose and 2×10^{-2} M $MgSO_4$. Then 10 μg/ml of DNase and 5 μg/ml of RNase were added and the mixture was homogenized in a Teflon glass homogenizer. The homogenized spheroplast suspension was diluted 200 fold with 0.05 M potassium phosphate buffer, pH 6.6 and incubated for 10 min at 37 C with vigorous shaking. After centrifugation at 15,000 rev/min for 30 min, the resulting pellets were washed at least four times by homogenization in cold 0.1 M potassium buffer, pH 6.6 and 10^{-2} M EDTA. After washing, the resulting membrane preparations were suspended in 8.0 ml of solution containing 0.01 M $MgSO_4$ and 20% sucrose. The suspensions were layered on the top of 60% sucrose (25 ml) in 0.1 M potassium phosphate buffer (pH 6.6) and 10^{-2} M $MgSO_4$, and centrifuged at 15,000 rev/min for 60 min in a Spinco L2 centrifuge using an SW27 swinging bucket rotor. The fractions at the top of the 60% sucrose were diluted 3 fold with potassium phosphate buffer, pH 6.6, and centrifuged at 28,000 rev/min for 60 min in a Spinco L2 centrifuge using a #30 angle rotor. The resulting pellets were sus-

pended in 8 ml of solution containing 0.1 M potassium phosphate (pH 6.6), 10^{-2} M $MgSO_4$ and 1.4×10^{-2} M glucose. This fraction did not contain more than 10^4 viable cells/ml.

10. *^{14}C-Proline uptake*

Membrane preparations at a concentration of 2 mg/ml in 0.1 M potassium phosphate buffer (pH 6.6) containing 10^{-3} M $MgSO_4$ and 1.4×10^{-2} M glucose were incubated for 15 min at 37 C. ^{14}C-Proline was then added and incubation at 37 C was continued. At intervals, 0.05 ml samples were diluted 20 fold with fresh buffer, rapidly filtered on Millipore HA filters (0.45 μ) and washed twice with 2 ml of buffer. The filters were dried and radioactivity was counted in an Aloka scintillation counter with scientillation fluid composed of 4 g of 2, 5-diphenyloxazole and 0.1 g of 1, 4-bis-2-(5-phenyloxazolyl)-benzene per liter of toluene.

11. *Chemicals*

Mitomycin C, mitomycin A (MTA), decarbamoyl mitomycin C (DCMTC), and 7-methoxymitosene (7-MMT) were kindly supplied by Kyowa Hakko Kogyo Co. Ltd, Tokyo. ^{14}C-Proline (specific activity 165 mCi/mmol) was obtained from Daiichi Kagaku Co., Tokyo.

RESULTS

1. *Genetic mapping*

The following two observations showed that the MTC-sensitive, UV-insensitive mutants (M5 and M11) are controlled by a mutation in a gene (designate as *mtcB*) other than that (*mtcA*) previously reported. First, strains M5 and M11 receiving F'13 which bears the *lac*$^+$ and *mtcA*$^+$ genes were still sensitive to MTC. Second, no mtc$^+$ recombinants were obtained among the pro$^+$ recombinants obtained on crossing BE591 (Cavalli Hfr) with M5 and M11, while about 90% of the recombinants were Mtc$^+$ in the cross of the same Hfr with mutant (M28 and M32), as previously described.

To locate the MTC sensitive gene in M5 and M11, the mutants were crossed with several types of Hfr strains. Among the 138 mtc$^+$, trp$^+$ recombinants selected after crossing BE-591 with M11 for 120 min, 24% were xyl$^+$, 22% were arg$^+$ and 21% were str^s. Among the mtc$^+$ thr$^+$ leu$^+$ colonies obtained on crossing AB313 with BE995, 42% were xyl$^+$. These results indicate that the mutation occurred close to the *xyl* and *arg* genes.

Among the 163 recombinants selected which were *thr*$^+$ *leu*$^+$ *xyl*$^+$ in the cross AB312$\times$BE-995 and the 131 recombinants selected which were *xyl*$^+$ *ilv*$^+$ *thy*$^+$ in the cross S3-1-1$\times$M11, none received donor *mtcB*$^+$ markers. When M11 was crossed with S21-1-1, none received donor *mtcB*$^+$ marker among of the 128 *his*$^+$ *ilv*$^+$ recombinants selected. Since AB312 and S3-1-1 transfer markers in the order *fda*, *argF*, *xyl* and *thr*, and S21-1-1 transfers them in the order *lys*, *thy and his*, the mutation lies somewhere between *fda* and *lys*. Therefore, it was assumed that the gene might be jointly transducible by phage P1 with markers located between 55 and 60 min on the *E. coli* chromosome. P1 phage prepared in M5 and M11 was used to transduce the MTC sensitive gene into a *metC*$^-$ recipient and selection was made for transductant receiving *metC*$^+$. As seen in

TABLE 2. *Frequency of occurrence of unselected donor markers in crosses of a mtcB mutant with Hfr strains*

Cross	Selected markers	No. of recombinants tested	No. of unselected donor markers						
			lac	*leu thr*	*arg*	*xyl*	*str*	*his*	*mtc*
BE591$\times$M11	trp$^+$, mtc$^+$	172	17	8	39	43	42	3	—
AB313$\times$BE995	thr$^+$, leu$^+$, mtc$^+$	250	—	—	0	104	—	7	—
AB312$\times$BE995	thr$^+$, leu$^+$, xyl$^+$	163	—	—	—	—	—	—	0
S21-1-1$\times$BE995	his$^+$, ilv$^+$	128	—	—	—	—	—	—	0

Table 3, about 15% of the *metC*$^+$ transductants were MTC sensitive, indicating that the mutation which controls MTC sensitivity in M5 and M11 lies close to the *metC* gene on the *E. coli* chromosome. From these results we concluded that there are at least two genes which control the MTC-sensitive, UV-insensitive characters of *E. coli*, the one (*mtcA*) is located at 12 min and the other (*mtcB*) is located at 58 min of the *E. coli* chromosome.

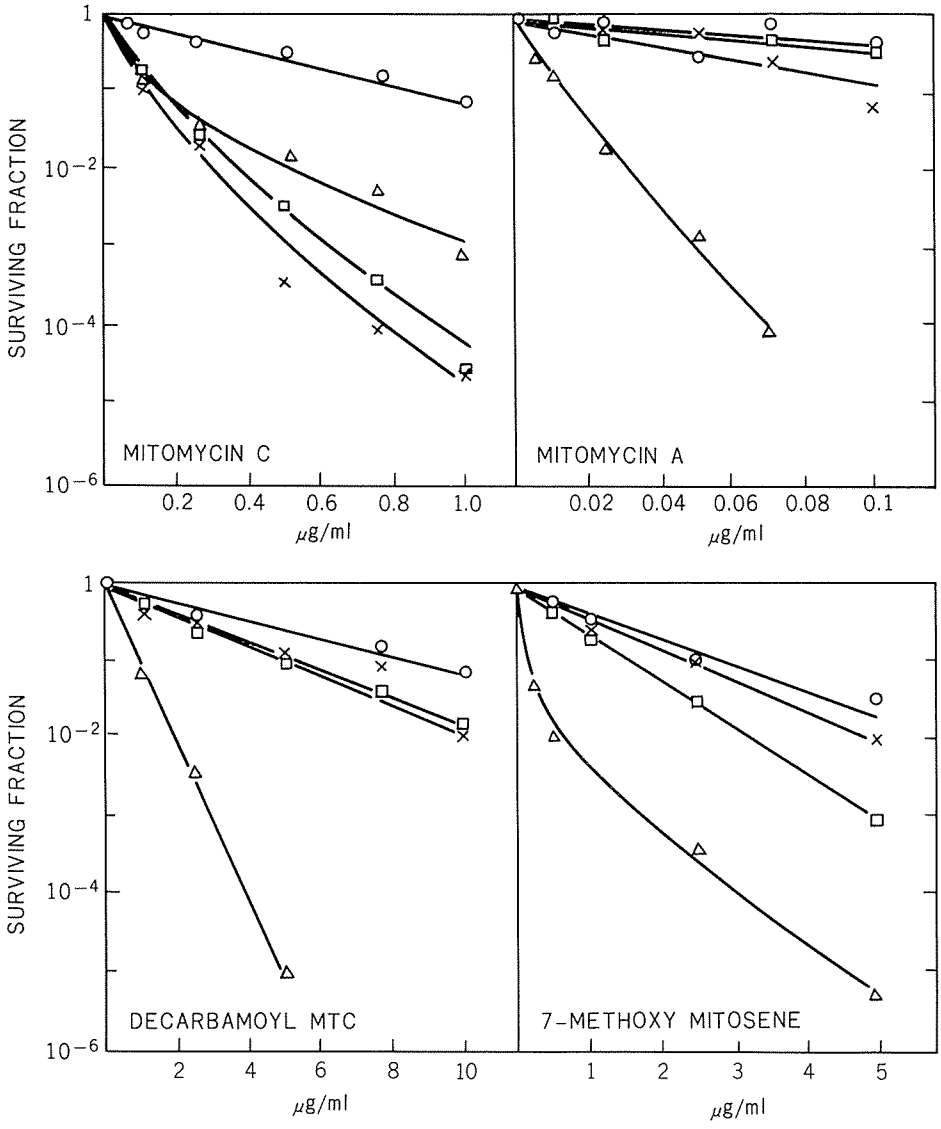

FIGURE 1. *Survival of Mitomycin C sensitive strains after exposure to various concentrations of mitomycin C, mitomycin A, decarbamoyl mitomycin C and 7-methoxymitosene.*
○——○, *AB1157;* △——△, *AB1885;* □——□, *M27;* ×——×, *M11.*

TABLE 3. *Mapping of the mtcB locus by P1-transduction*

Donor	Recipient	No. of *metC*$^+$ transductants analyzed	No. of *mtcB*
M5	AT2446	292	39
M11	AT2446	173	36

2. *MTC sensitivity*

Fig. 1 shows the survival of cells after exposure to mitomycins. Results obtained with the original strain, AB1157 and with a UV sensitive mutant, AB1885, are also included for comparison. As seen in Fig. 1A, both M11 (*mtcB*) and M27 (*mtcA*) were about 10 times more sensitive to the lethal action of MTC than the wild strain, since 37% survival was observed at a dose of about 0.05 μg/ml of MTC with M11 and M27 and at 0.6 μg/ml with the wild strain. The UV sensitive mutant is sensitive to the lethal actions of mitomycin A (MTA), decarbamoylmitomycin C (DCMTC) and 7-methoxymitosene (7-MMT), but *mtcA* and *mtcB* mutants are as resistant to these antibiotics as wild type bacteria. Since MTC and MTA are known to be efficient cross-linking agents (Iyer and Szybalski, 1963; Szybalski and Iyer, 1967), and DCMTC as well as 7-MMT is bound to DNA by monofunctional alkylation (Otsuji and Murayama, 1972), the mutants do not seem to be defective in the mechanism for removal of damaged DNA caused by mitomycins.

3. *Dye sensitivity*

During recombination experiments, it was noticed that both types of mutants formed tiny colonies on EMB agar plates. This suggested that the mutants might be sensitive to eosin or methylene blue. To test their sensitivity to dyes, bacteria grown to the log phase were diluted to 10^5 cells/ml in Penassay broth me-

TABLE 4. *Sensitivity of MTC sensitive strains to various kind of dyes*

Bacterial strains	mtc	Methylene blue						Toluidine blue						
		0	5	10	20	50	100	0	2	5	10	20	50	100
AB1157	+	+	+	+	+	+	−	+	+	+	+	+	+	−
M18, M27 M28	A	+	−	−	−	−	−	+	+	±	−	−	−	−
M5, M11	B	+	−	−	−	−	−	+	−	−	−	−	−	−
		Malachite green						Basic fuchsine						
		0	0.2	0.5	1	2	5	0	1	2	5	10	20	50
AB1157	+	+	+	+	+	+	−	+	+	+	+	+	+	−
M18, M27 M28	A	+	±	−	−	−	−	+	+	−	−	−	−	−
M5, M11	B	+	−	−	−	−	−	+	−	−	−	−	−	−
		Proflavine						Acridine orange						
		0	0	10	20	50		0	5	10	50	100		
AB1157	+	+	+	+	+	−		+	+	+	+	−		
M18, M27 M28	A	+	+	−	−	−		+	+	−	−	−		
M5, M11	B	+	+	−	−	−		+	+	−	−	−		

Bacteria grown in Penassay broth to approximately 2×10^8 cells/ml were diluted 100 fold and 0.1 ml volumes were inoculated into 2 ml of Penassay broth medium containing various concentrations of dyes. After about 18 hr incubation at 37 C without shaking, bacterial growth was recorded. {+: *full growth.* ±: *slight growth.* −: *no visible growth.*

dium containing various concentrations of dyes and then incubated at 37 C for about 20 hr. As seen in Table 4, growth of both mtcA and mtcB mutants was inhibited by lower concentrations of basic dyes than that of the wild type bacteria.

4. *SDS and DOC sensitivities*

It was reported that a mutant which was sensitive to proflavin also showed increased sensitivity to sodium dodecylsulfate (SDS) and the mutation was located near *lac* on the *E. coli* chromosome (Nakamura, 1965). MTC sensitive mutants were shown to be sensitive to proflavin, so it seemed that they might also be sensitive to SDS. When their growth in Penassay broth was tested, it was found that the *mtcB* and *mtcA* mutants both showed greatly increased sensitivity to SDS. Fig. 2A shows the survival of cells after exposure to various concentration of SDS. Both experiments show that the *mtcA* and *mtcB* mutants were more than 10 times more sensitive to SDS than wild type bacteria. Fig. 2B shows that mutants were also sensitive to the surface active substance, sodium deoxycholate (DOC).

This increased sensitivity of MTC sensitive mutants to basic dyes, SDS and DOC does not indicate increased sensitivity for all substances because the sensitivities of the mutants to EDTA, actinomycin D and the non-ionic detergent, Brij 58, were the same as those of wild type bacteria.

5. *Colicin E1 sensitivity*

De Zwaig and Luria (1967) reported that the colicin E1 tolerant mutant, tolVIII was, sensitive to methylene blue and DOC, and the mutation was located between *xyl* and *his* on the *E. coli* chromosome. Since these properties of the tolVIII mutant were very similar to those

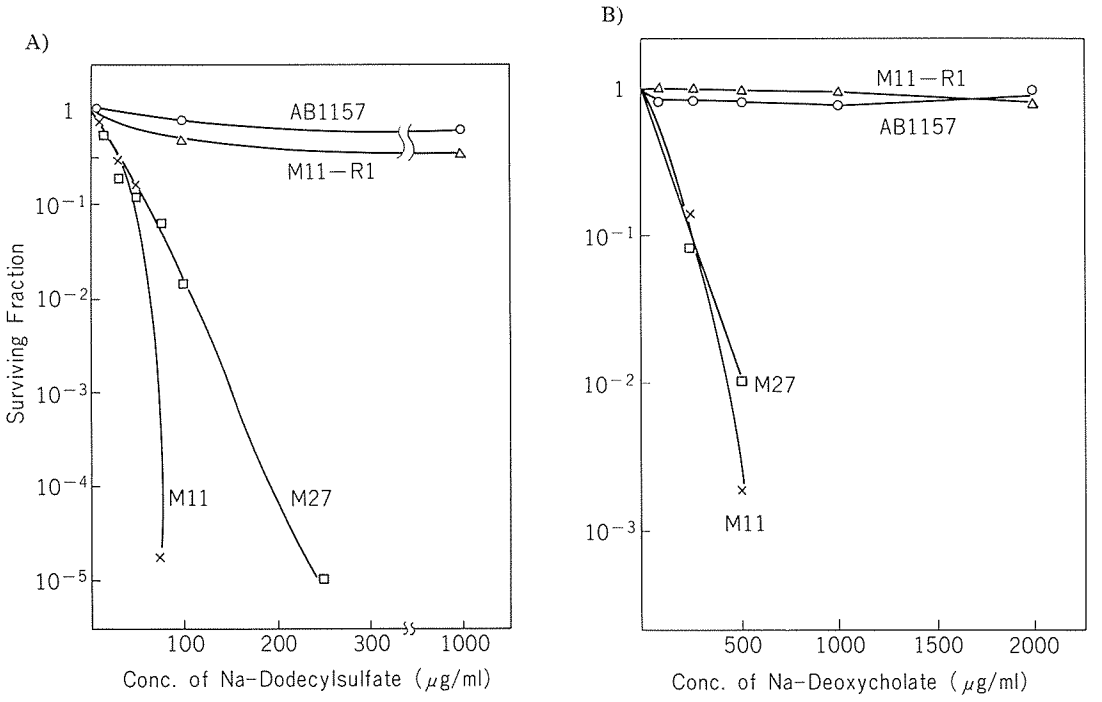

FIGURE 2. *Survival of mitomycin C sensitive strains after exposure to various concentrations of sodium dodecylsulfate (A) and sodium deoxycholate (B). Cells were incubated with SDS for 30 min at 37 C in Pennassay broth.* ○——○, *AB1157;* □- - -□, *M27;* ×——×, *M11;* △——△, *M11R-1.*

of the *mtcB* mutant, we tested the sensitivity of the mutant against colicins. When the mutant strain was streaked on nutrient broth medium containing colicin E1, E2, E3 or K, it was found that the *mtcA* mutant was as sensitive to E1, E2, E3 and K colicins as the wild type bacteria, while the *mtcB* mutant was resistant to E1, but sensitive to E2, E3 and K. Fig. 3 shows the survival of MTC sensitive strains after exposure to colicin E1, indicating that the *mtcB* mutant is more resistant to E1 colicin than the *mtcA* mutant and wild type bacteria.

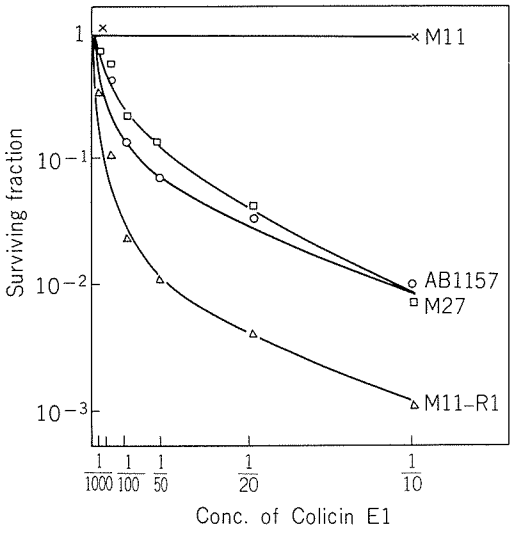

FIGURE 3. *Survival of mitomycin C sensitive strains after exposure to various doses of colicin E1. Bacteria grown in EM medium to the log phase were centrifuged, washed once and adjusted to* 3×10^8 *cells/ml in M buffer. After exposure to various concentrations of colicin at 37 C for 30 min, cells were diluted and plated for colony counts.*
○——○, *AB1157;* □——□, *M27;*
×——×, *M11;* △——△, *M11R-1.*

The above experiment suggested that mutation at the *mtc* locus is pleiotropic. If this be the case, a bacterial strain isolated as a mutant with one of the characteristics of the mtc mutant should have simultaneously acquired all the other phenotypic characteristics.

This was confirmed by the two following observations. First, isolated mutants, which were sensitive to SDS or methylene blue, proved to be sensitive to MTC also. Second, MTC resistant revertants from M11 (M11-R1) were found to have acquired all the other characteristics of the wild type; that is, the revertants were resistant to DOC and SDS, and sensitive to colicin E1. (Fig. 2 and 3).

6. *Binding of methylene blue with mutant strains*

The above results suggested that the mutants might have some alteration of the cell surface rendering it more permeable or easier to bind

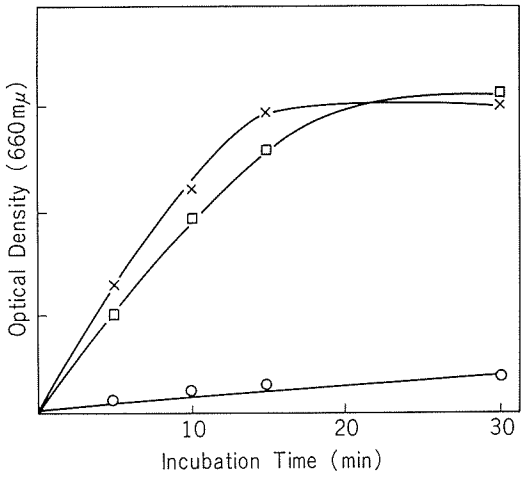

FIGURE 4. *Binding of methylene blue to mitomycin C sensitive strains.*
○——○, *AB1157;* □——□, *M27;*
×——×, *M11.*

with the test substances. To examine this possibility, cells grown to the logarithmic phase were incubated with methylene blue and aliquots of the samples were removed at intervals for measurement of the amount of dye bound to the bacterial cells. As seen in Fig. 4, the amount of dye bound to the mutant strains increased linearly for about 15 min and then reached a plateau at quite a high level, while only a small amount of dye combined with the wild type strain.

7. *Effect of colicin E1 on ^{14}C-proline uptake by membrane preparations*

Kaback and Stadtman (1966, 1968) reported that bacterial membrane vesicles, prepared by repeated centrifugation from spheroplasts disrupted osmotically, can incorporate amino acids and sugars. Using this technique, we tested the effect of colicin E1 on the ^{14}C-proline uptake by membrane preparations from wild type and mutant bacteria. Membrane vesicles prepared as described in the Materials and Methods showed a linear rate of ^{14}C-proline incorporation for about 45 min. When colicin E1 was added 5 min after the start of incubation and the reaction was stopped after 30 min, a greater inhibition of ^{14}C-proline uptake was observed in the membrane fraction of wild type bacteria than in that of the *mtcB* mutant strain (Table 5). Thus the ability of amino acid uptake by *mtcB* membranes is more resistant to the action of colicin E1 than that by wild type bacteria. This suggests that the surface structure of the *mtcB* mutant is altered making the cell refractory to the action of colicin E1.

TABLE 5. *Effect of colicin E1 on ^{14}C-proline uptake by membrane preparations*

Strain	Colicin E1 (unit/ml)	^{14}C-Proline (count/min) after 5 min	^{14}C-Proline (count/min) after 30 min	Inhibition (%)
AB1157	0	480	780	53
	1.5×10^{10}	480	620	
	0	460	870	70
	3.0×10^{10}	460	560	
M11	0	1300	2100	6
	1.5×10^{10}	1300	2050	
	0	1200	2080	40
	3.0×10^{10}	1200	1730	

Membrane preparations (2 mg/ml) were incubated in 0.1 M potassium phosphate buffer (pH 6.6) containing 10^{-3} M $MgSO_4$ and 1.4×10^{-2} glucose for 15 min at 37 C and then mixed with ^{14}C-proline (0.5 μCi/ml). 5 min later, colicin E1 was added and incubation was continued for 25 min.

DISCUSSION

This work shows that MTC-sensitive, UV-insensitive mutants can be divided into two groups, one controlled by a gene, *mtcA*, located close to the *lac* gene (Otsuji, 1968) and the other controlled by the *mtcB* gene located at about 58 min on the *E. coli* chromosome. Mutation at either the *mtcA* or *mtcB* gene has a pleiotropic effect; the mutants are also sensitive to basic dyes (Table 4), SDS and DOC (Fig. 2). These characteristics of the mutants suggested that the cells might have some defects of their surface structure. Increased binding of methylene blue with mutant cells (Fig. 4) and relatively high resistance to the inhibitory action by colicin E1 on ^{14}C-proline uptake by membrane vesicles of the *mtcB* mutant (Table 5) supported this assumption.

Both mutants, *MtcA* and *MtcB*, seem to have altered surface structures, but the changes in the surface which make the cell sensitive to MTC are not the same in the two mutants, since the former is sensitive to colicin E1, while the latter is not. The defect in the *mtcB* mutant is probably in the cell surface, preventing colicin E1 from transmitting its lethal action to the intracellular target, while the defect of the *mtcA* mutant seems to increase the sensitivity of the cells to MTC, DOC, SDS and basic dyes, but not to affect the action of colicin E1.

If *mtc* mutants are defective in a specific surface component which renders them sensitive to MTC, then the question arises of how a mutation in the locus causes an alteration in the cell surface. A preliminary experiment using a temperature dependent *mtcB* mutant suggested that some component of the cell surface in the mutant may not be made in a normal form or may be made in an altered form at the elevated temperature (T. Higashi and N. Otsuji, unpublished).

Recently it was reported that a colicin E1 tolerant mutation (*tolC*) occured at about 58 min on the *E. coli* chromosome (Whitney, 1971). The physiological characters and map

position of the *tolC* mutation resemble to those of the mutation of *mtcB* described in this paper (De Zwaig and Luria, 1967; Bhattacharyya et al., 1970; Whitney, 1971), so it seems likely that *tolC* and *mtcB* may be identical, and that MTC sensitivity and colicin E1 tolerance are pleiotropic properties of this one gene.

Both MTA and DCMTC are biologically active derivatives of mitomycin group antibiotics. The MTC-sensitive mutant, however was not sensitive to these two compounds. The $-NH_2$ group at position 7 in the MTC molecule is replaced with $-OCH_3$ in MTA and the $-CH_2OCONH_2$ group at the 10a position is replaced by $-CH_2OH$ in DCMTC (Fig. 5). Studies on the relation of the peculiar structure of the mitomycin molecule with the sensitivity of mutant strains should give important information on the mechanism of MTC-sensitivity in *E. coli*.

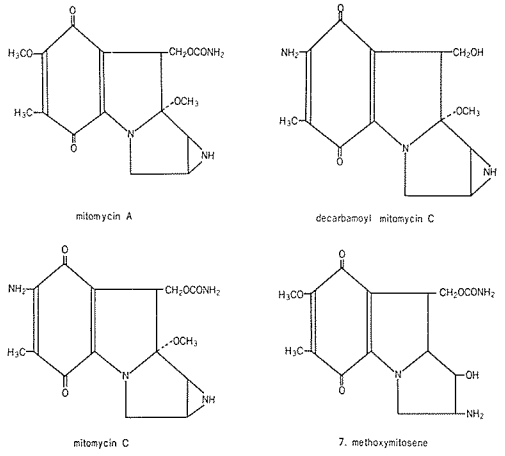

FIGURE 5. *Chemical structures of mitomycins.*

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